TOBACCO INDUSTRY RESEARCH COMMITTEE

CONFIDENTIAL

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A Study of Structure-Activity Relationships Among Drugs Which Affect Nicotine-Sensitive Physiological Mechanisms

A total of 22 experiments have been performed in barbitalized cats to determine the effect of agents which impair ganglionic transmission on the output of acetylcholine from the superior cervical ganglion. In all experiments, the superior cervical ganglion was perfused, essentially as first described by Kibjakov, using a warm, oxygenated, Ringer's solution containing neostigmine; the content of "transmitter substance" in the effluent from the ganglion was assayed as acetylcholine using the isolated rat ileal strip preparation we have described in an earlier report.

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In five cats, death of the animal or injury to the ganglion precluded carrying out our anticipated experiments. Seven cats were used to develop the surgical and mechanical techniques used in the work. Four cats were used exclusively to study the vascular dynamics and behavior of the ganglion under the conditions of the experiment. In 5 experiments, in four of which ganglionic blocking drugs were administered, collection and bioassays were performed on effluent from the ganglion; in two of the three experiments with nicotine, behavior of both the donor preparation and of the assay preparation permitted adequate study of the effects of the drug on "transmitter" output.

Many of our results had not been anticipated, on the basis of our studies of the previous publications concerning this preparation; some of these unanticipated results are summarized in Figures 1 and 2, in which data from representative experiments are graphed; results of the two successful experiments with nicotine are graphed in Figures 3 and 4.

In contrast to our impression gained from the literature, we were unable to isolate the circulation to the ganglion from the systemic circulation. This fact is documented in Figure 1. Surgical isolation of the ganglion, complete at time "A" in the figure, never resulted in obtaining a blood-free effluent from the ganglion. In Figure 1 are plotted the hemoglobin concentrations of systemic venous blood and of effluent samples as a percent of initial systemic blood hemoglobin concentration (hemoglobin was determined spectrophotometrically in hemolyzed samples). Perfusion of the surgically "isolated" ganglion with Ringer's solution caused progressive dilution of systemic blood and a slight decrease in hemoglobin concentration of the effluent. Ligation of the major cervical vessels on the side contralateral to the infusion ("B" in Figure 1) permitted obtaining a lower hemoglobin concentration in the effluent than in the systemic blood. Only death of the cat ("C" in Figure 1) made it possible to obtain a hemoglobin-free effluent. Simultaneous with achievement of a blood-free effluent resistance to perfusion (mean pressure head/output of constant output pump) decreased. Although death of the cat prevented influx of blood into the perfusate, it did not prevent efflux of perfusate into the systemic vasculature, as indicated by the ratio (effluent (ml/min)/pump output (ml/min)) which, except during the period of asphyxia, was uniformly much less than one. At the end of the experiment shown in Figure 1, it was found that only about 17% of the total volume infused had been recovered in the effluent.

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All experiments with ganglionic blocking agents, designed to measure the effects of the agent on "transmitter" output, have been and will be performed on cats which are dead, systemically, in order to facilitate collection of effluent from the ganglion alone.

Although preganglionic nerve stimulation had no consistent effect on flow of perfusate through the ganglion (occasionally such stimulation was followed by the appearance of minute amounts of blood in the previously clear effluent) the administration of nicotine in doses known to prevent ganglionic transmission was followed regularly by changes in ganglionic perfusion. Such changes are represented in Figure 2. Retrograde intra-arterial injection of nicotine (100 mcg) caused a rise in resistance to perfusion and, in this case, a relative decrease in the amount of effluent (this is a reflection of an absolute decrease in the amount of effluent). These changes occurred regardless of ligation of the contralateral cervical vessels and regardless of the death of the cat. Communication between the perfusion system and circulatory system is demonstrated graphically in the remarkable change in systemic blood pressure produced by the first intra-arterial injection of nicotine.

Preganglionic nerve stimulation was not found to have consistent effects in increasing the output of substance spasmogenic to the rat ileum (Fig. 3 and 4). This is in contrast to data reported previously in the literature. However, like the activity of acetylcholine and like the activity of materials detected in ganglionic effluent by other investigators, spasmogenic activity in effluents obtained in our experiments is abolished by boiling the effluent and by atropinization of the assay preparation.

In Figures 3 and 4 are shown the results obtained in two experiments involving retrograde intra-arterial administration to the ganglion of doses of nicotine (100 mcg) which prevent ganglionic transmission. In both experiments, drug administration was followed by a decrease in output of spasmogen in the effluent; concomitant to this change was a decrease in response of the nictitating membrane to preganglionic stimulation. In both experiments, administration of nicotine was followed by an increased resistance to perfusion; in both experiments this increase in resistance was accompanied by a moderate increase in the fraction of perfusate recovered as effluent, although the absolute amount of effluent decreased in both experiments. It is as if injection of nicotine closed to perfusion vascular beds previously open and in communication with the total vascular system of the animal.

Comparison of Figures 3 and 4 indicates that perfusion is not necessarily accompanied by a progressive decline in spasmogen output. In both cases, however, injection of nicotine produced a sudden decrease in spasmogen output. It is conceivable, in light of the data, that the effect observed after nicotine was the result only of a failure of efflux from a nonganglionic site (but richer in spasmogen than efflux from the ganglion, per se) to gain access to the total effluent, and that the failure of the nictitating membrane to respond and the decrease in spasmogen output were related coincidentally rather than causally. In refutation of this point of view can be mentioned only that one experiment

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in which the usual experimental procedure was intentionally carried out on a cat in which the ganglion as well as the body was dead, indicated that no spasmogen was leeched from the tissues by perfusion, or that it was produced in quantities much less than 10^{-10} grams/minute.

With the reservations introduced by the effects of nicotine on the vasculature of the perfused tissues, our results are consistent with our original hypothesis that nicotine exerts its effects in impairing ganglionic transmission by decreasing the output of transmitter substance. Future experiments must include:

1. Additional studies to confirm the observed effects of nicotine on spasmogen output.

2. Experiments with other agents such as morphine or tetraethylammonium ion, which should affect transmitter output as nicotine is presumed to do, and experiments with hexamethonium ion which we anticipate will prevent ganglionic transmission without altering spasmogen output.

3. Experiments involving such procedures as surgical extirpation of the ganglion, or using drugs which decrease spasmogen output without affecting blood vessels in the area perfused, or alter blood vessel diameter without changing spasmogen output, in order to evaluate the significance of changes in vascular resistance in producing results such as we have obtained so far.

4. Experiments designed to determine the chemical nature of the spasmogen assayed in our preparation, or to identify it certainly with acetylcholine or other hypothesized chemical mediators of synaptic transmission.

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FIGURE 1 PERFUSION OF CAT SUPERIOR CERVICAL GANGLION

AT "A", PERFUSION BEGUN; AT "B", LIGATION OF CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS; AT "C", CAT KILLED BY ASPHYXIATION. TOTAL VOLUME INFUSED: 403 ml; TOTAL EFFLUENT FROM TRANSVERSE POSTERIOR FACIAL VEIN: 69 ml.

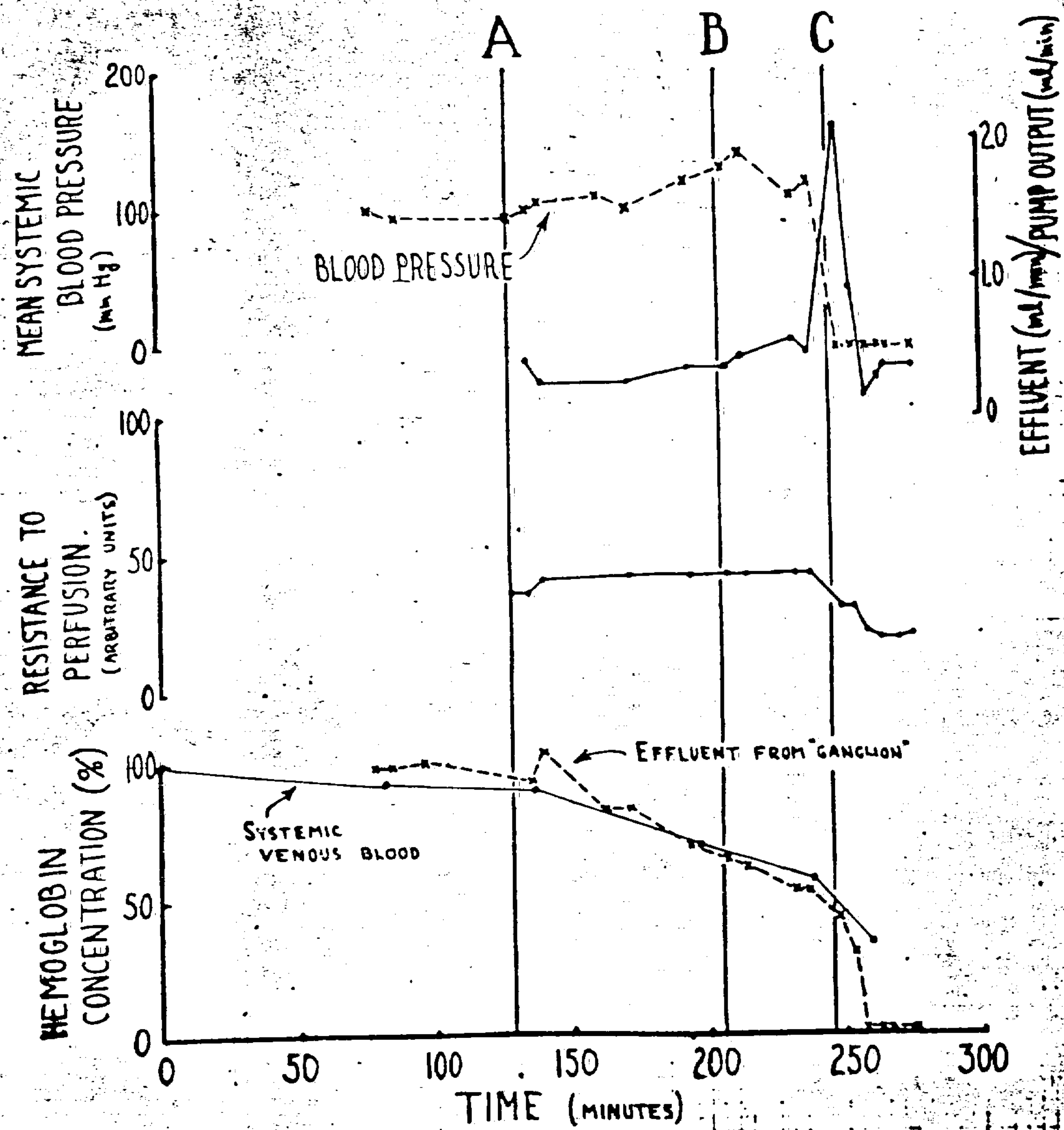

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FIGURE 2: PERFUSION OF CAT SUPERIOR CERVICAL GANGLION

AT "A", PERFUSION BEGUN; AT "B", CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS LIGATED AND CAT KILLED BY ASPHYXIATION.

AT "N", NICOTINE, 100 mcg, ADMINISTERED TO GANGLION BY INTRA-ARTERIAL INJECTION.

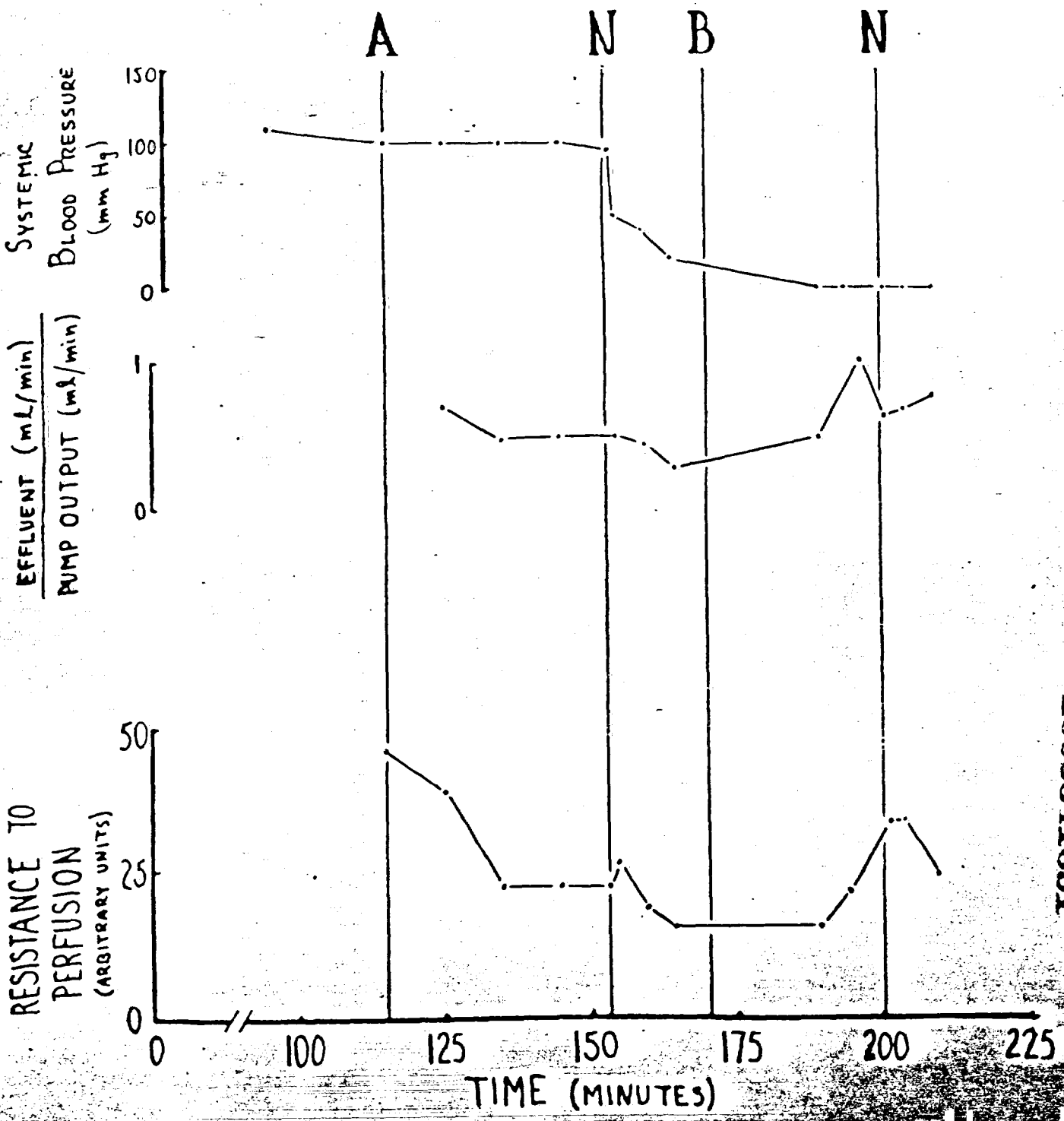

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FIGURE 3 EFFECT OF NICOTINE ON RATE OF RELEASE OF SPASMOGEN FROM PERFUSED SUPERIOR CERVICAL GANGLION OF CAT. CLOSED CIRCLES SHOW RESULTS OF ASSAYS IN DUPLICATE ON EFFLUENT OBTAINED DURING PRE-GANGLIONIC NERVE STIMULATION; OPEN CIRCLES INDICATE RESULTS OBTAINED WITHOUT STIMULATION. HEAVY HORIZONTAL BARS INDICATE GEOMETRIC MEANS OF REPLICATE ASSAYS AND DURATION OF COLLECTION PERIOD OF EFFLUENT. CAT WAS DEAD OF ASPHYXIATION AND CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS HAD BEEN LIGATED. ASSAY OF SPASMOGEN WAS PERFORMED ON ISOLATED RAT ILEAL STRIP.

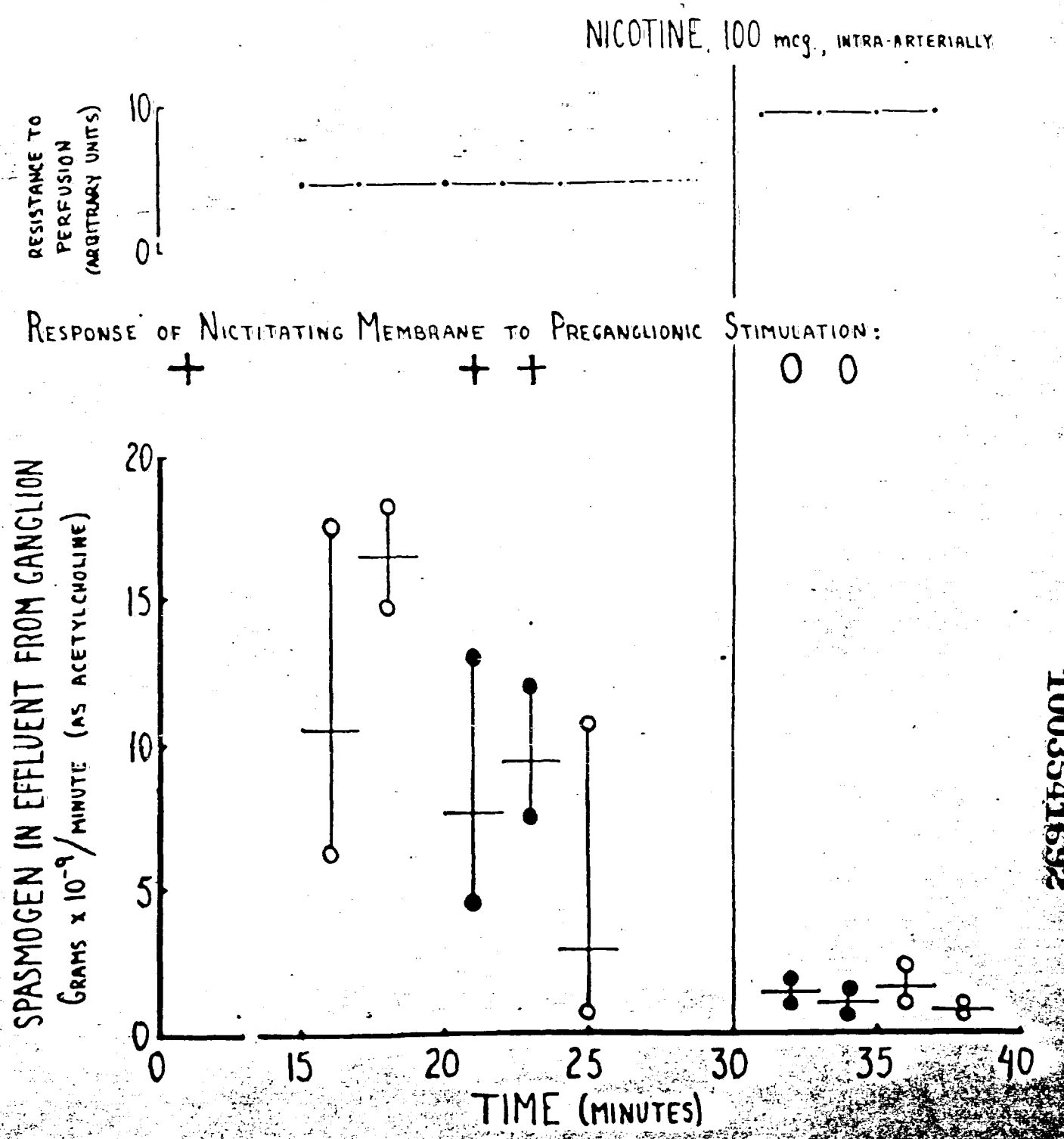

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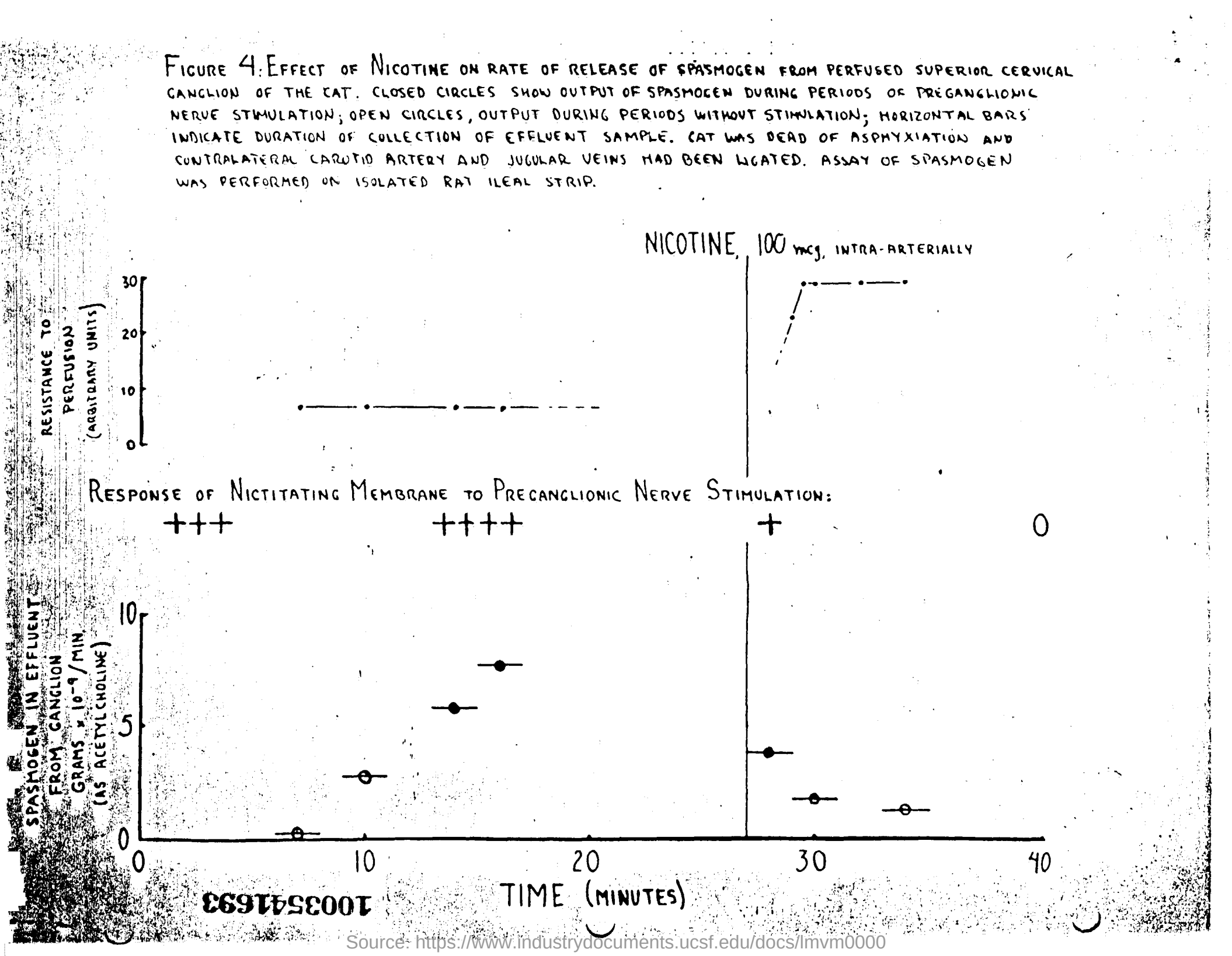
FIGURE 4: EFFECT OF NICOTINE ON RATE OF RELEASE OF SPASMOGEN FROM PERFUSED SUPERIOR CERVICAL GANGLION OF THE CAT. CLOSED CIRCLES SHOW OUTPUT OF SPASMOGEN DURING PERIODS OF PREGANGLIONIC NERVE STIMULATION; OPEN CIRCLES, OUTPUT DURING PERIODS WITHOUT STIMULATION; HORIZONTAL BARS INDICATE DURATION OF COLLECTION OF EFFLUENT SAMPLE. CAT WAS DEAD OF ASPHYXIATION AND CONTRALATERAL CAROTID ARTERY AND JUGULAR VEINS HAD BEEN LIGATED. ASSAY OF SPASMOGEN WAS PERFORMED ON ISOLATED RAT ILEAL STRIP.



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